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Antinociceptive activity of various solvent extracts of *Maerua angolensis* DC stem bark in rodents

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Abstract

Various parts of *Maerua angolensis* notably the leaves, roots and stem barks are used to reduce pain and also in the management of psychosis, epilepsy, and arthritis in traditional medicine. In this study, a preliminary test was performed to determine the most active plant part on the hydroethanolic extracts in the abdominal writhing test in mice. Subsequently, the stem bark, the most active plant part, was extracted with petroleum ether, ethyl acetate or hydroethanol to obtain three extracts which were tested for analgesic activity in the formalin test in rats. Animals were grouped into groups of 5 and the extracts were administered orally. Diclofenac and morphine were used as reference analgesic agents while normal saline was used for control. The leaf, root and stem bark extracts (30, 100 and 300 mg/kg) significantly ($P<0.0002$) and dose-dependently reduced abdominal writhes induced by acetic acid with the stem bark extract being more active. In the formalin test, all the extracts (3, 10 and 30 mg/kg) significantly ($P<0.0006$) and dose-dependently reduced the frequency and time spent in biting/licking of injected paws in both the neurogenic and inflammatory phases induced by formalin. The petroleum ether extract was most active in neurogenic while ethyl acetate was most active in inflammatory phase. Results justify the use of the plant parts in ethnomedicine for the management of various painful conditions.

Keywords: *Maerua angolensis*, Antinociception, Writhing, Formalin test.

Introduction

Maerua angolensis (family Capparidaceae) is a tropical plant that is widespread in the savannah area of tropical Africa to South Africa and Swaziland.[1, 2] It is a tree whose size varies from medium to big and growing up to 10 – 20 meters high. It is commonly found growing in bush and rocky areas but planted on graves in Nupe area of Nigeria. Maerua angolensis has a long history of use in traditional medicine to manage various painful conditions in Nigeria and other West African countries. Various parts of the plant notably the leaves, roots and stem barks are claimed to reduce pain and are used to manage psychosis, epilepsy, diabetes, peptic ulcer, diarrhea and arthritis in the traditional medicine.[3, 4] Phytochemical screening of the methanolic stem bark extract revealed the major constituents as saponins, tannins, flavonoids, alkaloids and glycosides.[3] The median lethal dose of the stem bark extract of the plant in mice orally and intraperitoneally showed the stem bark of the plant to be relatively safe.[5] Lack of scientific proof of analgesic efficacy of this plant claimed by the traditional healers called for the study. In this study, a preliminary test was performed to determine the most active plant part on the hydroethanolic extracts in the abdominal writhing test in

mice. Subsequently, the stem bark, the most active plant part, was extracted with petroleum ether, ethyl acetate or hydroethanol to obtain three extracts which were tested for analgesic activity in the formalin test in rats.[6]

Materials and Methods

Plant materials

Fresh leaves, roots and stem barks of Maerua angolensis were collected at Samaru campus of Ahmadu Bello University, Zaria–Nigeria (August, 2012) and were identified by Dr. Kofi Annan of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi–Ghana. A voucher specimen (KNUST/FP/12/051) was kept at the herbarium of the Faculty.

Preparation of extracts

The leaves, roots and stem barks were separately shade-dried, pulverized into coarse powder and 100 g extracted with 1 L 70% v/v ethanol in water (hydroethanol) by cold maceration for 4 days. Each extract was filtered and then concentrated in a rotary evaporator to give greenish, brownish and yellowish syrupy mass respectively for the leaf, root and stem bark. These were dried in hot air oven giving yields of 17.6, 7.1 and 10.4% w/w in that order. In addition to these extracts, 4 kg of the powdered stem bark was sequentially extracted for 4 days with 10 L of petroleum ether, ethyl acetate and hydroethanol (in order of increasing polarity) by cold maceration. The extracts obtained were treated as described above to give yields of 2.23, 3.87 and 7.8% w/w respectively for the petroleum ether (MABPEE), ethyl acetate (MABEAE) and hydroethanol (MABHAE). For drug administration, all extracts were freshly prepared as suspension in normal saline containing 2% Tween 40 (vehicle) before use.

Animals

Sprague–Dawley rats (190 – 200 g) and ICR mice (20 – 25 g) of either sex were used in the study. All animals were housed in groups of five in stainless steel cages (34 x 47 x 18 cm) with softwood shavings as bedding in the animal facility of the Department, KNUST, with free access to food and water and were maintained under normal laboratory conditions of humidity, temperature (25 ± 1 ºC) and a 12 h/12 h day/night cycle. The investigation conforms to the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH No. 85 – 23, revised 1996) and The Institutional Animal Ethics Committee approved all procedures. In all the experimental studies each group consisted of 5 animals.

Drugs and chemicals

The following drugs and chemicals were used: Acetic acid and formalin (BDH, Poole, England), diclofenac sodium (Troge Medical GmbH, Hamburg, Germany), morphine hydrochloride (Phyto–Riker, Accra, Ghana). All drugs used in the nociceptive tests were dissolved in normal saline.

Writhing test

The leaf, root or stem bark extracts of *Maerua angolensis* at 3 dose levels (30, 100 and 300 mg/kg, orally), diclofenac sodium (10 mg/kg, intraperitoneally) as reference analgesic agent or normal saline (10 ml/kg, intraperitoneally) for control were administered to groups of mice. Acetic acid (0.6% v/v) was given (10 ml/kg, intraperitoneally) 1 h after oral and 30 min after intraperitoneal administration to all mice. The number of abdominal constrictions (writhing) per 5 min segments for 15 min counted 5 min after acetic acid injection was captured by camcorder and tracked with the help of JwatcherTM software. A significant reduction in the number of acetic acid–induced abdominal constrictions by any treatment compared with control treated mice was considered as an antinociceptive response.[7, 8]

Formalin test

The formalin test was carried out as described by Tjolsen and Fischer.[9, 10] Rats were given MABPEE, MABEAE or MABHAE at 3 dose levels (3, 10 and 30 mg/kg, orally), morphine hydrochloride (1, 3 and 10 mg/kg, intraperitoneally) as reference analgesic agent or normal saline (10 ml/kg, intraperitoneally) for control. Formalin (5%) was injected (10 µl) into the dorsal surface of the right hind paw 1 h after oral and 30 min after intraperitoneal administration to all rats to induce pain.[11, 12] The amount of time spent licking/biting the injected paw was measured and taken as an indication of pain.[13] Pain response per 5 min segments for 1 h was scored starting immediately after formalin injection. Average nociceptive score for each time block was determined as product of frequency and time spent in biting/licking the injected

paw. The first phase of the nociceptive response normally peaks 0 – 5 min and the second phase 15 – 30 min after formalin injection corresponding to the neurogenic and inflammatory pain responses respectively.[14, 15]

Data analysis

Data were expressed as mean ± standard error of the means (SEM) per group. Statistical differences between control and treated groups were tested by two–way (treatment x time) repeated measures analysis of variance (ANOVA) with Bonferroni's post hoc test, differences between other means were by one-way ANOVA with Newman–Keuls post hoc test. The ED_{50} (dose of extract or drug necessary to reduce the response by 50% relative to the control value) and 95% confidence intervals values were determined by using nonlinear regression (three-parameter logistic). GraphPad Prism 6.00 for Windows (GraphPad Prism Software, San Diego, CA, USA) was used for all statistical analyses. Differences were considered significant at $P<0.05$.

Results

Effect of hydroethanol leaf, root and stem bark extracts on the writhing test

Fig. 1A, 1B and 1C shows that hydroethanol leaf, stem bark and root extracts of *Maerua angolensis* respectively, given orally 1 h prior to testing, produced significant ($F_{4, 20} = 99.58$, $P<0.00001$, $F_{4, 20} = 113.80$, $P<0.0001$ and $F_{4, 20} = 9.18$, $P<0.0002$) and dose-related inhibition of the acetic acid-induced abdominal constrictions in mice. The inhibition was 82 ± 5%, 73 ± 4% and 62 ± 16% for the highest dose of hydroethanol leaf, stem bark and root extracts respectively. Similar effect was observed in mice pre-treated by the non steroidal anti–inflammatory drug (NSAID), diclofenac used as a reference analgesic agent.

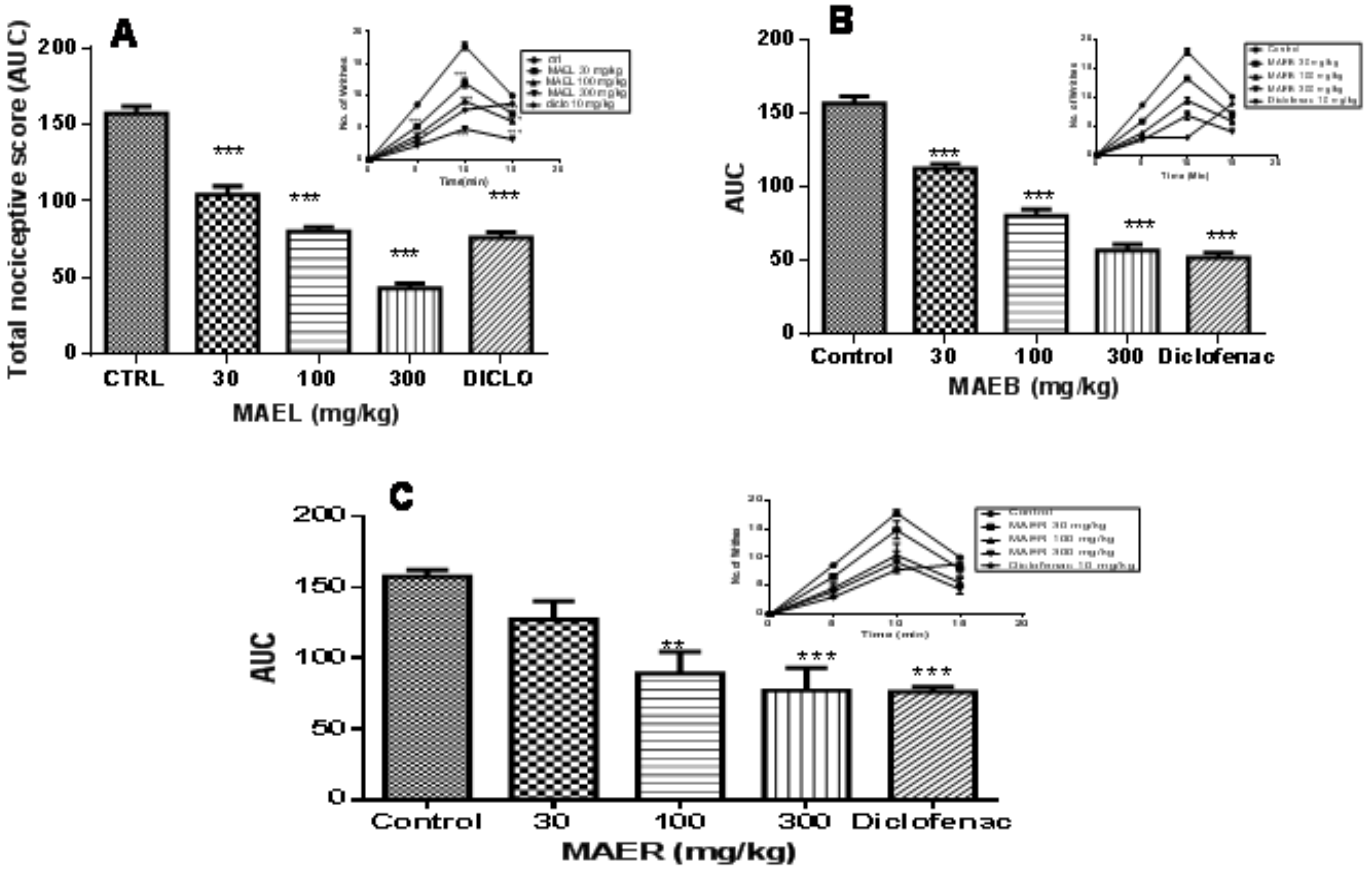

Figure 1: Effect of aqueous ethanol leaf (MAEL) (A), stem bark (MAEB) (B) and root (MAER) (C) extract of *Maerua angolensis* or diclofenac against acetic acid-induced writhing response in mice. Each column represents the mean of five mice, and the error bar indicates the SEM. Asterisk denotes the significance level compared with control groups (one-way ANOVA followed by Newman Keuls post hoc test): **P<0.01, and **P<0.001

The ED_{50} for hydroethanol leaf, root and stem bark extracts in the writhing test (Table 1) showed the hydroethanol stem bark extract was more potent in preventing the nociception caused by acetic acid. Thus, the stem bark extraction with various solvents (in order of increasing polarity) was chosen for further studies in formalin test with independent groups of animals.

Table 1: ED_{50} values for hydroethanol leaf, root and stem bark extracts of Maerua angolensis in the writhing test

Treatment	ED_{50} (mg/kg) in writhing
Hydroethanol leaf extract	52.31 ± 0.09
Hydroethanol root extract	56.25 ± 0.36
Hydroethanol stem bark extract	49.45 ± 0.08

Values are expressed as mean ± SEM, (n = 5)

Effect of MABPEE, MABEAE and MABHAE on the formalin-induced nociception

The antinociceptive effect of MABPEE (Fig. 2A), MABEAE (Fig. 2B) and MABHAE (Fig. 2C) examined in the formalin model of persistent pain showed significant and dose-dependent decrease in paw licking time on both the neurogenic ($F_{3, 16} = 19.43$, $P<0.0001$, $F_{3, 16} = 13.89$, $P<0.0001$ and $F_{3, 16} = 9.86$, $P<0.0006$) and the inflammatory phase ($F_{3, 16} = 30.22$, $P<0.0001$, $F_{3, 16} = 24.29$, $P<0.0001$ and $F_{3, 16} = 17.04$, $P<0.0001$) when compared with vehicle treated control rats. In a similar manner, morphine hydrochloride (Fig. 2D) pretreatment resulted in a distinct dose–dependent reduction of response time in the early ($F_{3, 16} = 36.78$, $P<0.0001$) and late ($F_{3, 16} = 34.76$, $P<0.0001$) phases of formalin-induced licking.

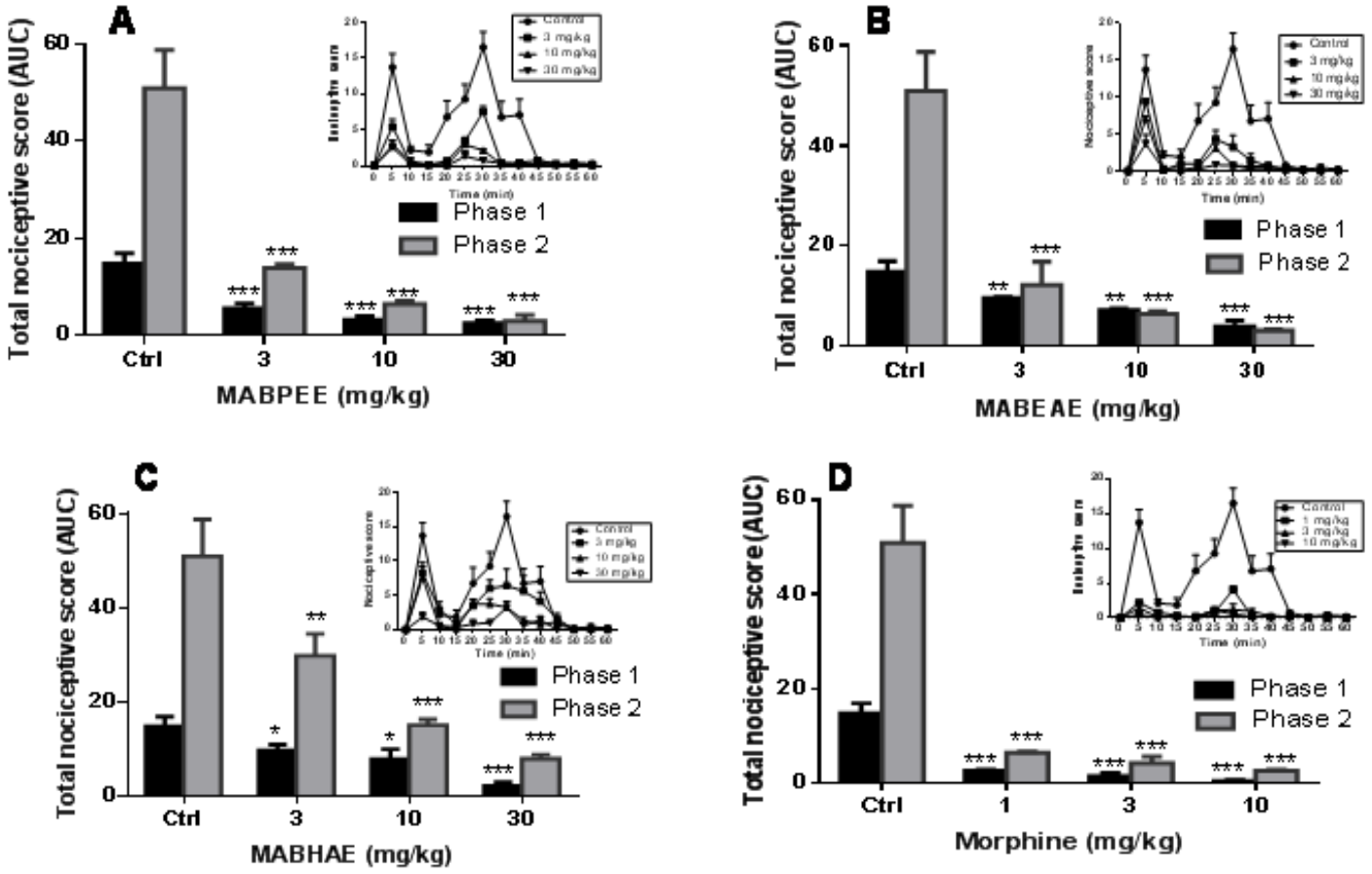

Figure 2: Effect of petroleum ether (MABPEE) (A), ethyl acetate (MABEAE) (B) and aqueous ethanol (MABHAE) (C) bark extract of *Maerua angolensis* or morphine (D) on formalin-induced nociception. Each column represents the mean of five rats, and the error bar indicates the SEM. Asterisk denotes the significance level compared with control groups (one-way ANOVA followed by Newman Keuls post hoc test): *P<0.05, **P<0.01, and **P<0.001

The calculated mean ED_{50} values for the Antinociceptive effects of MABPEE, MABEAE, MABHAE and morphine in the formalin test (Table 2) showed MABPEE was more potent in inhibiting the neurogenic pain and MABEAE more potent in blocking pain emanating from inflammation. Morphine was, however, most potent on both the neurogenic and the inflammatory phase of the formalin-induced licking.

Table 2: ED_{50} values for MABPEE, MABEAE, MABHAE and morphine in the formalin test

Treatment	ED_{50} (mg/kg) in early phase	ED_{50} (mg/kg) in late phase
MABPEE	1.12 ± 0.20	0.99 ± 0.06
MABEAE	4.61 ± 0.15	0.79 ± 0.20
MABHAE	9.89 ± 0.28	3.81 ± 0.13
Morphine	0.19 ± 0.12	0.09 ± 0.03

Values are expressed as mean ± SEM, (n = 5)

Discussion

Maerua angolensis is traditionally used in the treatment of pain however, lacking a pharmacological evaluation for its analgesic effect. The outcome of our investigation demonstrates that the leaves, stem barks and roots of *Maerua angolensis* have significant analgesic activity against chemical-induced pain model in mice. In acetic acid-induced writhing test, the plant leaves, roots and stem barks extracts suppressed the pain sensation in a dose dependent manner however; stem bark extract was more potent. Similar effects were observed in mice pre-treated by the NSAID, diclofenac used as a reference analgesic agent. This may be a further confirmation to the usefulness from time immemorial of some medicinal plants such as *Maerua angolensis* for the management of pain. It is also an indication that all the extracts are readily absorbed following oral administration.

Acetic acid-induced abdominal writhing test is a model of acute persistent nociception and a typical model for inflammatory pain in which acetic acid is used as the algogenic agent. Acetic acid when injected intraperitoneally induces visceral pain in the animals via stimulation of primary afferent sensory Ad and C nerve fibers[16] and generally the test is popular in detecting peripheral analgesic agents.[17-19] Abdominal constriction test has good sensitivity and is capable of detecting antinociceptive compounds at doses that may be inactive with other antinociceptive tests. Related studies have established that acetic acid indirectly induces the release of pro-inflammatory prostanoids (prostaglandins) which in turn cause the production of prostanoid-dependent pain causing molecule bradykinin, important in the mechanism of pain transduction in primary afferent nociceptors.[20-21] Additionally, prostaglandins sensitize peripheral pain through activation of EP receptors present on the peripheral terminals of sensory neurons.[22, 23] Acetic acid also liberates sympathetic nervous system mediators that stimulate the nociceptive neurons, all of which are sensitive to non–steroidal anti-inflammatory drugs and opioid analgesics.[18, 24, 25] Diclofenac, a non-opioid analgesic inhibits the production and release of prostaglandins accompanied by reduction in the abdominal writhes. However, an opioid analgesic such as morphine is effective in abolishing the acetic acid-induced pain in an opioid way at periphery.[26, 27] Our results indicated that the leaf, root and stem bark extracts of *Maerua angolensis* could reduce the number of writhing in the animal model of pain similar to diclofenac, implying that it had antinociceptive effect which may be due to inhibition of synthesis and/or release of pro-inflammatory prostanoids peripherally. Antinociception may have also occurred spinally through inhibition of pro-inflammatory mediators-mediated central sensitization. This activity probably is due to the presence of flavonoids, alkaloids, saponins, glycosides and tannins as reported in literature.[3] The drawback of this test is that it has poor specificity (drugs such as muscle relaxants used as 'adjuvant' in pain management exhibit antinociceptive activity in this test), leaving span for the misinterpretation of results.[28] Furthermore, the results of this writhing test alone cannot establish whether the antinociception was central or peripheral. Notwithstanding, in order to further study this, formalin licking test in rats was performed to complement the writhing test.

Formalin test is the most predictive model of acute tonic pain and has an advantage of discriminating pain into central and/or peripheral components. It has been reported that formalin–induced persistent nociception in mice paws produced a marked biphasic licking response.[14] The first phase (early or neurogenic phase) of the nociceptive behavior (paw licking/biting response) after formalin injection which starts immediately after injection might be due to direct stimulation of nociceptors such as transient receptor potential ankyrin1 (TRPA1) and transient receptor

potential vanilloid1 (TRPV1) receptors by formalin or involvement of substance P and bradykinin in nociceptors sensitization, while the second phase (late or inflammatory phase) which appears a little later is taken to be due to a combination of an inflammatory reaction in the peripheral tissue and changes in central processing.[9] Hunskaar[14] have established that central analgesics, such as opioids (morphine) inhibit both phases, while peripherally acting agents, such as steroids (hydrocortisone) and NSAIDs (diclofenac) curb mainly the late phase. A significant and dose–dependent antinociceptive effect was evident for the tested MABPEE, MABEAE and MABHAE against both neurogenic and inflammatory pain behavior caused by formalin injection in rats similar to morphine. Analgesic effect in the second phase of the formalin test is predictive of antihyperalgesic activity of our extracts in neuropathic pain models.[11, 29, 30] It is possible that the mechanism of action of the solvent extracts could be peripherally similar to morphine by blocking substance P or bradykinin known to be involved in nociceptors sensitization or by inhibiting TRPA1 or TRPV1 receptors at the spinal site in phase 1. It could also be centrally by blocking pro-inflammatory pain mediators known to be involved in phase 2 or by inhibiting nociceptive effects of transmitters like glutamate which act as descending facilitators. In inflammatory pain, arachidonic acid is converted into a variety of intermediate substances with the help of endogenous enzymes, cyclooxygenase-1 and -2 which is elevated resulting in an increase in prostaglandin E2 production. Melgaard[31] suggested that prostaglandin E2, may mediate an increase in nitric oxide (NO) production resulting to increase vasodilatation and capillary permeability leading to edema and sensitization of pain fibers. It is likely the solvent extracts are acting by inhibiting the cyclooxygenase, thereby decreasing prostaglandin production which otherwise would cause pain. The antinociceptive activity could be as a result of the presence of flavonoids and tannins among other constituents as reported in literature.[3] These substances are known to possess potent analgesic properties.[32-34] More so, flavonoids potently inhibit prostaglandins, which are pro–inflammatory signaling molecules. Flavonoids also inhibit phosphodiesterases involved in cell activation[32, 35] the effect of which is on the biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to sites of injury. Whatever it is, the exact mechanism of antinociception of these extracts needs to be established.

Conclusion

In summary, *Maerua angolensis* extracts (leaf, root and stem bark) displayed antinociceptive effect in acetic acid-induced abdominal constrictions in mice with the bark extract being more potent, whereas, the petroleum ether, ethyl acetate and aqueous ethanol stem bark extracts of the plant showed analgesic effect on both the neurogenic and the inflammatory phases of formalin-induced paw licking in rats with the petroleum ether being more potent in the neurogenic pain and ethyl acetate more potent in the inflammatory pain. Our results support the traditional uses of this plant in neurogenic and inflammatory pain.

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